The Effects of various Chemical Agents upon the Starch-converting Power of Taka Diastase

By Karl F. Kellerman

The importance which the study of enzymes has acquired in the last few years has brought the chemist and physiologist into even closer relationship than that existing before. Furthermore, in studying the general problems of fermentation, both as regards the effects of various enzymes on each other, and in some cases their action in relation to the growth and nutrition of the organism containing them, it has seemed to the writer that as a foundation for accurate work a knowledge of the effects of physical and chemical conditions upon the enzymes is absolutely necessary.

For certain diastases the effects of various physical conditions have been rather carefully worked out, but results relative to the action of chemical agents upon these diastases seem more or less meager and scattered. It has been attempted to study in a systematic way the effect of a considerable number of chemical agents upon diastatic action, and the results of the work are given in this paper.

Taka diastase was chiefly used as the subject of the following experiments, and is here reported upon in detail, on account of its uniformly rapid action and its great keeping qualities. However, most of the work has been repeated, using malt diastase instead of taka, and occasional references will be made to the former.

This taka diastase is the Japanese Saké ferment, prepared from the fungus *Eurotium Oryzae*, and now sold commercially for treatment of amylaceous dyspepsia. This enzyme is a little less sensitive to the presence of foreign substances, and its action, though at first more rapid, is not so complete as that of malt diastase.* That is, there is a greater percentage of starch not converted in the case of taka diastase, even after long-continued action, although the conversion of the other portion of the starch is much more rapid.

* Stone & Wright, Journal of the American Chemical Society, 20: 167, 681.

Some rather interesting results were obtained in a preliminary series of experiments. It was found that, the amounts of starch and diastase being constant, the converting power of the enzyme became more and more rapid with the concentration of the solution of starch, or starch paste. The solutions of starch varied from 3 per cent., which is rather viscous, to 0.5 per cent., which is very watery. For determination of the sugar present, all were diluted to 0.5 per cent. to make the physical conditions exactly the same; they were then heated rapidly in an autoclave to 110° C., to destroy the enzyme, and finally the relative amounts of sugar in the different bottles were determined volumetrically by means of Fehling's copper-alkaline-tartrate solution. In each determination, 2 c.c. of the freshly mixed standard was used, diluted to one half concentration, and the solution in question was added drop by drop to the boiling copper solution until the copper was completely precipitated. The end reaction was tested with potassium ferrocyanide in the presence of acetic acid. The relative amounts of sugar in the different solutions can be very accurately determined in this way, the amount of sugar in each bottle varying inversely as the amount of the solution necessary to cause complete precipitation.

Table I shows in detail the variations in speed of starch transformation due to dilution. The ordinates represent the number of cubic centimeters necessary to reduce 2 c.c. of Fehling's solution, and the first four abscissae represent the four different per cents. of starch experimented with. Thus the line starting at the seventh ordinate and 3 per cent. abscissa shows that 7 c.c. of the 3 per cent. starch solution were necessary to reduce 2 c.c. of Fehling's solution; of the 1.5 per cent. starch solution, 8.8 c.c. were necessary, etc.

As Duclaux* has carefully worked out, the conversion of starch into sugar is most rapid soon after action begins, and decreases slowly at first, then more and more rapidly until most of the starch is converted and action ceases.

The last six abscissae in Table I show my results. Here the abscissae represent the number of hours the enzyme was allowed to act before determination.

* Duclaux, Annales de l'Institut Pasteur, **12**: 96.

The effect of increasing the amount of diastase, the starch solution remaining constant, is to increase the rapidity of transformation, though not in proportion to the increase in the amount of enzyme. This is shown in Table I, in the three series that were allowed to act seven, five and three hours respectively. Here the abscissae represent the amounts of a 0.25 per cent. solution of taka diastase used in each case.

In the following experiments * the different chemical agents were prepared each in 100 c.c. of a sterilized 1 per cent. potato starch solution, and placed in thoroughly sterilized and well-stoppered bottles. Usually seven different substances, each with four different concentrations, were tested at the same time. These, together with four to six check bottles, containing 100 c.c. of pure starch solution — to make certain the relative determination of the effects of the various compounds — constitutes a series.

To each bottle of such a series was added 2 c.c. of a 0.25 per cent. solution of taka diastase. All were then placed in a thermostat and kept at 43° C. for about twelve hours. The thermostat was light-proof, and therefore precluded any inaccuracy due to the breaking down of the enzyme by light, or its acceleration caused by the action of light filtered through glass.† After incubation the entire series was placed in an autoclave and heated rapidly to 110° C. This temperature effectually destroys the diastase and any zymogen that might be present, as a preliminary experiment clearly showed.

Care in handling and setting up a series, and sterilization of everything used avoided any inaccuracy due to bacterial action, as indicated by numerous checks.

The relative amounts of sugar in the different bottles were determined volumetrically against 2 c.c. of Fehling's solution, as before. Whenever the chemical agent present interfered with accurate determinations, either by hydrolyzing the starch or by interfering with the reaction of Fehling's solution, the agent was removed or so changed as to be innocuous before heating to destroy the enzyme. Thus acids were neutralized, the copper salts precipitated out as hydroxides, etc.

* In all cases in these experiments Eimer & Amend's C. P. materials were used

† J. R. Green, Transactions Royal Society, **188**, B: 167.

The transformation was allowed to proceed until the check solutions were a little more than half converted into sugar; this corresponds to about the fourth ordinate in the tables.

The chemical agents used are given in fractions of the normal. The normal solution was made by dissolving as many grams of the salt as correspond to its molecular weight in less than one liter of the sterile 1 per cent. starch paste, and the solution then made up to exactly 1,000 c.c. by the addition of the starch paste.

The chemical agents experimented with fall naturally into four classes—the mineral acids and organic acids, the salts of these acids, the alkalies, and the metals.

It will not be attempted to give a complete summation of literature, but such will be cited as seem to bear directly on the subject.

It has been noted by Baranetsky,* Chittenden & Griswold,† and Effront ‡ that strong solutions of mineral acids destroy diastase and weak ones accelerate its action. Cohnheim § records no effect for moderate concentrations of HCl, and Langley & Eves || report that "the slightest trace" (0.015 per cent., about $n/240$) of HCl is very injurious.

Table II shows the results of my work. In this table, as in all following, the abscissae represent the strengths of the chemical agents and the ordinates represent the amount of the solution tested necessary to reduce 2 c.c. of Fehling's solution. "Trace" means that so slight an amount of sugar was present that over 15 c.c. of the solution would be necessary to reduce the Fehling's solution.

At a concentration of $n/10$ all the acids completely checked enzymetic action. At $n/100$ chromic acid gave complete inhibition; the other mineral acids allowed a slight action at $n/100$ and gave a marked acceleration at a dilution of $n/1000$. Sulphuric acid gave the most marked results, the amount of sugar produced being almost double that of the check. Chromic acid still gave a

* Baranetsky, Die Starkeumbildenden Fermente in den Pflanzen. (Leipzig, 1876.)

† Chittenden & Griswold, American Chemical Journal, 3 : 205.

‡ Effront, Comptes Rendus, 115 : 1324.

§ Cohnheim, Archiv für Pathologie, Anatomie und Physiologie (Virchow), 28 : 241.

|| Langley & Eves, Journal of Physiology, 4 : 18.

slight inhibition. At a dilution of $n/10{,}000$ there was a slight acceleration with hydrochloric and nitric acids, and none with the others. Malt diastase requires a $n/500$ dilution before any starch is converted, and at $n/1{,}000$ requires nearly 12 c.c. to reduce the 2 c.c. of Fehling's solution. Acceleration does not set in until a dilution of nearly $n/5{,}000$ is reached.

Among the organic acids, Detmer * for citric, Krauch † for salicylic, and Kjeldahl ‡ for lactic, butyric, formic, salicylic, acetic and others, record an acceleration for weak dilutions of the acids. §

* Detmer, Zeitschrift für physiologische Chemie, 7 : 1.

† Krauch, Landwirtschaftliche Versuchstation, 23 : 77.

‡ Kjeldahl, Zeitschrift für das gesammte Brauwesen, 3 : 179. 1880.

§ In this connection, perhaps Dr. Leffmann's paper, "Digestive Ferments with Especial Reference to the Effects of Food Preservatives" (Journal of the Franklin Institute, 147 : 97), should be mentioned.

Dr. Leffmann was working merely to find what antiseptics were injurious to enzymetic action, and records only inhibitory effect. He used only the iodine test to determine the presence of unconverted starch and dextrines, and hence it is impossible to accurately compare his results with mine. It seems, however, that in his experiments with taka diastase, tartaric and citric acids gave a decided inhibition, while in my work, at supposedly corresponding concentrations these acids were almost without effect.

It will be seen on the other hand that he notes no injurious action due to formalin, using 3 c.c. of formalin and, as far as I can gather from his paper, 50 c.c. of 1 per cent. starch. This corresponds to about $n/3$ formalin, which, as will be seen later in my paper, I find to be very injurious indeed. This brings out the fact that the iodine test is entirely inadequate for determinations where formalin is present. If pure starch solution in the presence of formalin is allowed to stand a few hours, it will give a dextrine reaction, and if the action is allowed to continue twelve to twenty-four hours, or if the starch and formalin are boiled together, no starch or dextrine color reaction can be obtained with iodine. Yet if these solutions are treated with NH_4OH to break the formalin down to hexamethyleneamine $(CH_2)_6N_4$, and any excess of ammonia neutralized, iodine will again give the typical starch blue, and Fehling's will give no reaction. Now if the iodine is added immediately upon the addition of formalin to pure starch solution, so that the starch is colored blue, then the formalin does not affect the color even upon standing twenty-four hours; while if this blue starch solution containing formalin is boiled, it loses its color, even after cooling, and upon the addition of more iodine gives now the red, or so-called erythrodextrine reaction. The action of the formalin is upon the starch, for even if iodine and formalin have been boiled together, the iodine will produce as good a blue as before.

These results hold both for starch paste and fresh starch grains. The latter show no difference under the microscope, even after a treatment with formalin sufficient to preclude their coloring with iodine. It seems to me, therefore, that formalin either has some physical action upon the starch or else forms some unstable compound, comparable to the supposed starch-iodine compound to which is due the blue of the starch test, but being very much more unstable.

By referring to Table II it will be seen that in my work the general effects seem much the same for these acids as for the mineral acids. Malic and acetic acid, however, after giving the usual acceleration near $n/1{,}000$, gave a marked inhibition as the dilution was carried further. This same peculiarity is shown by sulphuric and citric acids to a very much less extent. Malt diastase shows the same phenomenon. For instance, with acetic acid the acceleration occurs in the region of $n/12{,}500$ dilution, while $n/62{,}500$ distinctly retards conversion.

For taka diastase, malic acid did not stop conversion at $n/10$, and at $n/100$ gave a marked acceleration and still greater at $n/1{,}000$. Then at $n/10{,}000$ the inhibition was very marked. Acetic acid also did not stop conversion at $n/10$ concentration, but gave no acceleration until a dilution of $n/2{,}500$ was reached, and at dilutions of $n/12{,}500$ and $n/62{,}500$ gave almost as marked inhibition as malic acid.

The work of Gillott * on the inversion of maltose by tartaric, citric and oxalic acids suggested that the apparent increase in the amount of sugar in my experiments with the dilute solutions of these acids might be due to the inversion of the maltose into dextrose. To determine this a $n/10$ solution of each of the acids used was allowed to act on a one-per-cent. solution of C. P. maltose for twenty-four hours at a temperature of 43° C. The solutions were then all neutralized and the coefficients of their reducing power determined and compared with that of the untreated one-per-cent. solution of maltose. There seemed to be very little, if any, difference between them. It would seem, therefore, that the action of the weak acid is a true acceleration, and that the return to the normal action of the ferment at greater dilutions of the acid is due merely to weakening of the stimulus. There remains the chance that the effect of the acids during the breaking up and hydrolysis of the starch into maltose may cause the starch to be changed in part to grape sugar instead of maltose, and thus cause the increased reduction of Fehling's solution.

Among the workers who have dealt with the effects of salts and other bodies upon diastases, Chittenden and Ely † noted the

* Gillott, Bull. Assoc. Belg. Chim. 13: 80, 119.

† Chittenden & Ely, Journal of Physiology, 3: 327.

increased energy of the enzyme in the presence of one-per-cent. peptone, and also in the presence of sodium chloride, while dibasic sodium phosphate is not favorable. Effront * divided the favorable salts into three groups: salts of aluminum, phosphates and various amides, as asparagin.

From Tables III and V it may be seen that the diastase was much less sensitive to these salts and organic bodies than it was to acids. A normal solution was used as the maximum, instead of one tenth normal. The differences in their effects between sodium, potassium, calcium and magnesium salts of the same acid were greater than the different salts of any one of those bases, which seems here to indicate that the cation is more important than the anion. The calcium and magnesium salts seem more injurious than sodium and potassium, with the exception of the monobasic calcium phosphate, which gives practically no action at $n/10$ concentration, and a marked acceleration at dilutions of $n/100$ and $n/1{,}000$. Potassium bichromate gave a slight check at $n/128$, and none at $n/256$. Calcium sulphate and magnesium phosphate are so insoluble that they are given on Table III merely in terms of saturation, "*Excess*" standing for a saturated solution at 43°, "*Sat.*" for a saturated solution at 23°. Sodium chloride, potassium nitrate and potassium phosphate gave the greatest acclerations. Malt diastase closely follows the action of the taka diastase and shows no especial point of variation from it in the presence of these chemical agents.

A large number of investigators have tried peptone and asparagin, and all have noted a decided acceleration. Other organic compounds, however, seem to act more as do the salts of the mineral acids; thay have less marked effects, and are more irregular.

As seen in Table V, sodium acetate and ammonium citrate are the most injurious of those used, giving a marked inhibition at a concentration of $n/2$; their injurious action decreases rapidly to some intermediate point, and they accelerate starch conversion at dilutions of $n/100$ and $n/1{,}000$. Sodium formate and sodium lactate act very slightly either to retard or accelerate transformation. Potassium tartrate and sodium acetate gave the most acceleration of the organic series. Formalin inhibits markedly; even up to

* Effront, *l. c.*

$n/1{,}000$ nearly halving the amount of sugar. Chloral hydrate is injurious, though a slight acceleration is given at a dilution of $n/1{,}000$. Peptone accelerates most at 2.5 per cent., and still markedly at $\frac{1}{20}$ per cent. Asparagin accelerates strongly at $n/20$, and still slightly at $n/1{,}000$.

Many observers note the detrimental action of alkalies on certain diastases. Chittenden & Ely * note a decrease of one third in the amount of sugar due to 1 per cent. of sodium carbonate. Langley & Eves † found 0.0015 per cent. sodium carbonate checks starch transformation, and potassium hydroxide checks more still. They note that the rate of decrease in the effect of weaker concentrations of alkalies is slow compared to acids.

Without exception, in my work the alkalies seemed detrimental; slightly so even up to $n/10{,}000$ dilution. Sodium, potassium and ammonium seem slightly less injurious than calcium. In no case was there any acceleration in the presence of an alkali. (Compare Table IV.)

The metals are in general injurious. (Compare Table IV.) Iron allowed no action at $n/10$; being more injurious than copper, which did not entirely stop conversion until a $n/4$ concentration was reached. Copper sulphate and copper chloride acted very much alike, both giving a slight acceleration at $n/10{,}000$. Silver was very detrimental indeed. No conversion took place below $n/10{,}000$, and only a slight amount at $n/100{,}000$. Zinc nitrate and barium chloride gave a peculiar curve, similar to that of some of the acids, but at $n/100$ barium chloride just reached the normal, and inhibited at $n/1{,}000$, while zinc nitrate accelerated transformation at $n/100$ and inhibited action at $n/1{,}000$.

The work of Clark ‡ on the decrease of toxicity of mercuric chloride by the addition of other chlorides suggested that a similar series of experiments be tried on taka diastase.

In my experiments a $n/5{,}420$ solution of mercuric chloride was used, and to that varying amounts of calcium chloride were added. The results were very striking, as is shown in Table VI. Here the plain line represents the action of the solution of constant

* Chittenden & Ely, *l. c.*

† Langley & Eves, *l. c.*

‡ Clark, Journal of Physical Chemistry, **5**: 289.

strength of mercuric chloride to which various dilutions of other chlorides were added. The crossed line represents the action of the various dilutions of the chlorides alone.

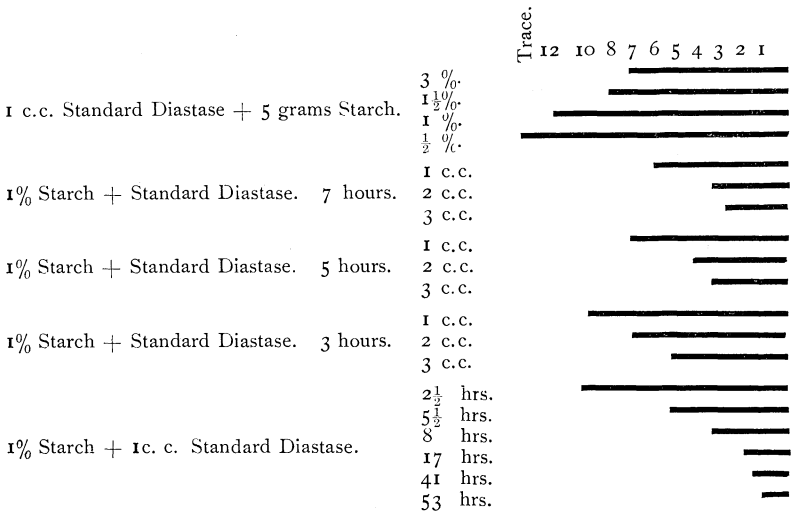
At the strong concentrations of the calcium, the check solutions containing only calcium were only slightly more rich in sugar than the solutions containing the mercury also. From $n/10$ to $n/1{,}000$, however, while calcium alone gave a slight increase above the normal starch transformation, the solutions containing mercury also were rapidly checked, until at $n/1{,}000$ dilution of calcium the $n/5{,}420$ mercuric chloride completely checked starch transformation.

A similar series of experiments was made with barium chloride substituted for calcium, and with as marked results. Then a series was carried through using sodium chloride, with four concentrations of mercuric chloride. Here the amount of sodium chloride was so constantly in excess that the sodium and sodium-mercury lines run fairly near together on the chart. It seems, however, to indicate an increase of injurious effect at the last dilution.

In conclusion I beg leave to acknowledge my indebtedness to Dr. B. M. Duggar, assistant professor of plant physiology, and Professor George F. Atkinson, professor of botany in Cornell University, for constant encouragement and every courtesy and assistance in the prosecution of this work.

TABLE I.

SHOWING EFFECT OF VARIATION OF TIME, CONCENTRATION OF DIASTASE AND CONCENTRATION OF STARCH

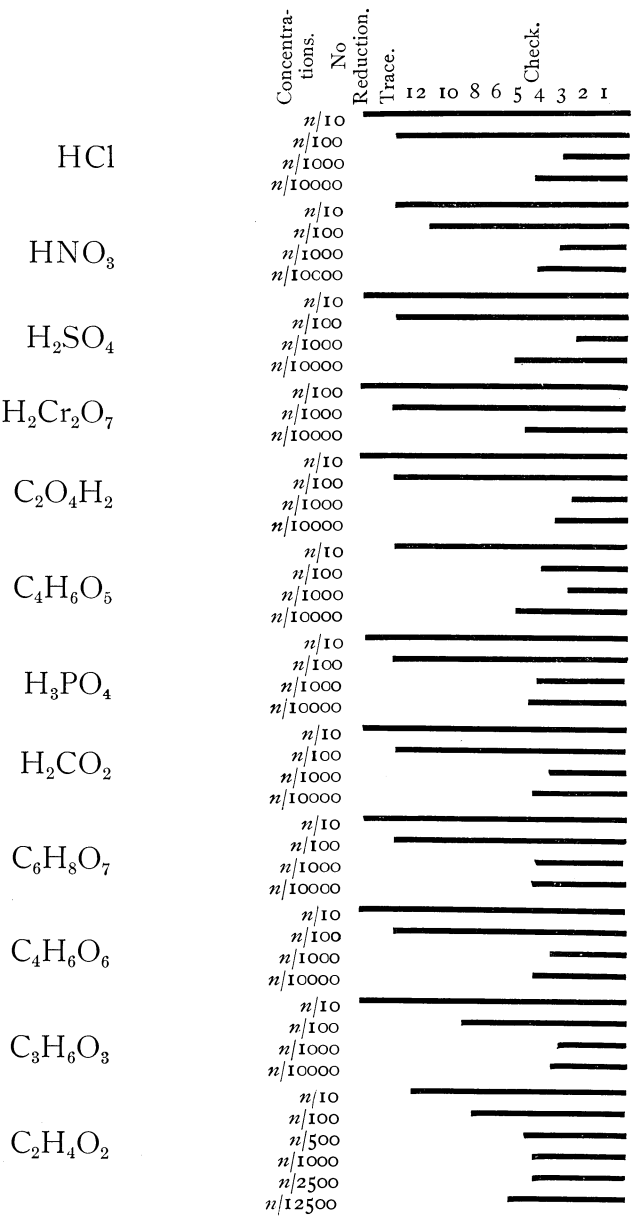


The numbers above the ordinates represent the number of cubic centimeters of the solution tested necessary to completely reduce 2 c.c. of Fehling's solution; therefore the shorter the heavy line, the greater has been the starch transformation in that particular case.

The numbers by the abscissae represent percentage of starch, amount of diastase, and variation of time, respectively.

TABLE II.

SHOWING EFFECTS OF VARIOUS ACIDS



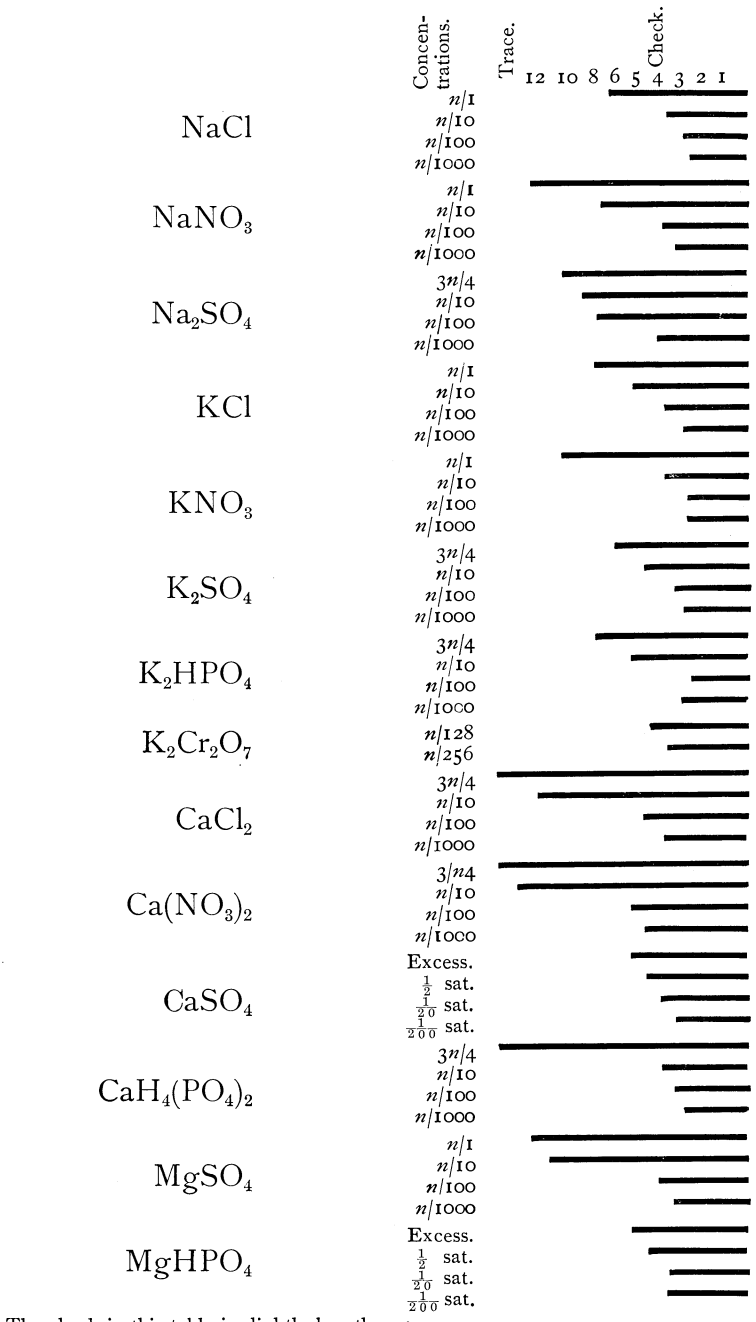
The ordinates represent the number of cubic centimeters of the solution tested necessary to reduce 2 c.c. of Fehling's solution, "Trace" representing an amount of over 15 c.c.

The abscissae represent the fraction of the normal concentration of the chemical agent used.

The check between 4 and 5 represents the number of cubic centimeters of the check solutions, containing only pure starch and diastase, necessary to reduce 2 c.c. Fehling's solution.

TABLE III.

SHOWING EFFECTS OF VARIOUS SALTS



The check in this table is slightly less than 4.

TABLE IV.

SHOWING EFFECTS OF VARIOUS ALKALIES AND METALS

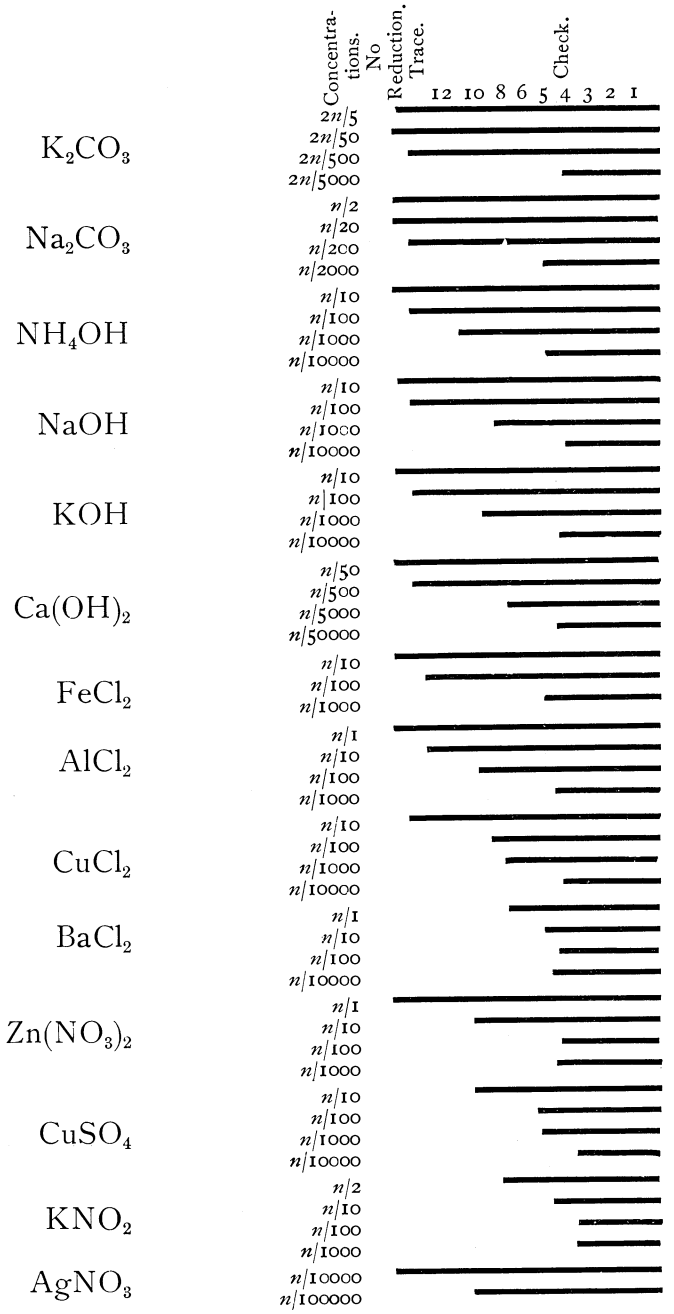


TABLE V.

SHOWING THE EFFECTS OF VARIOUS ORGANIC COMPOUNDS

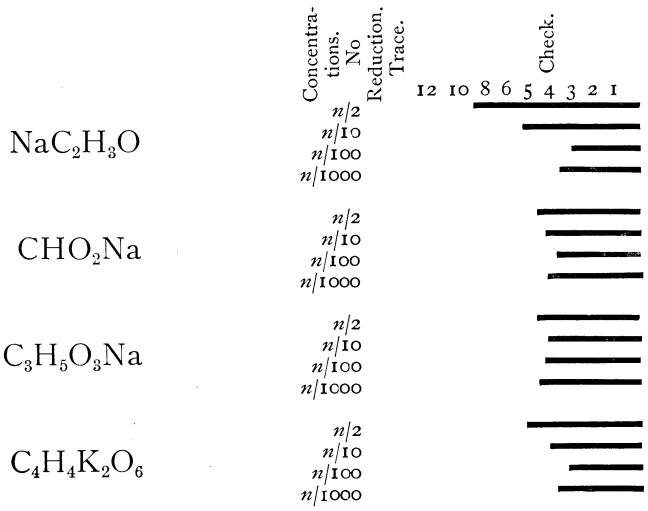
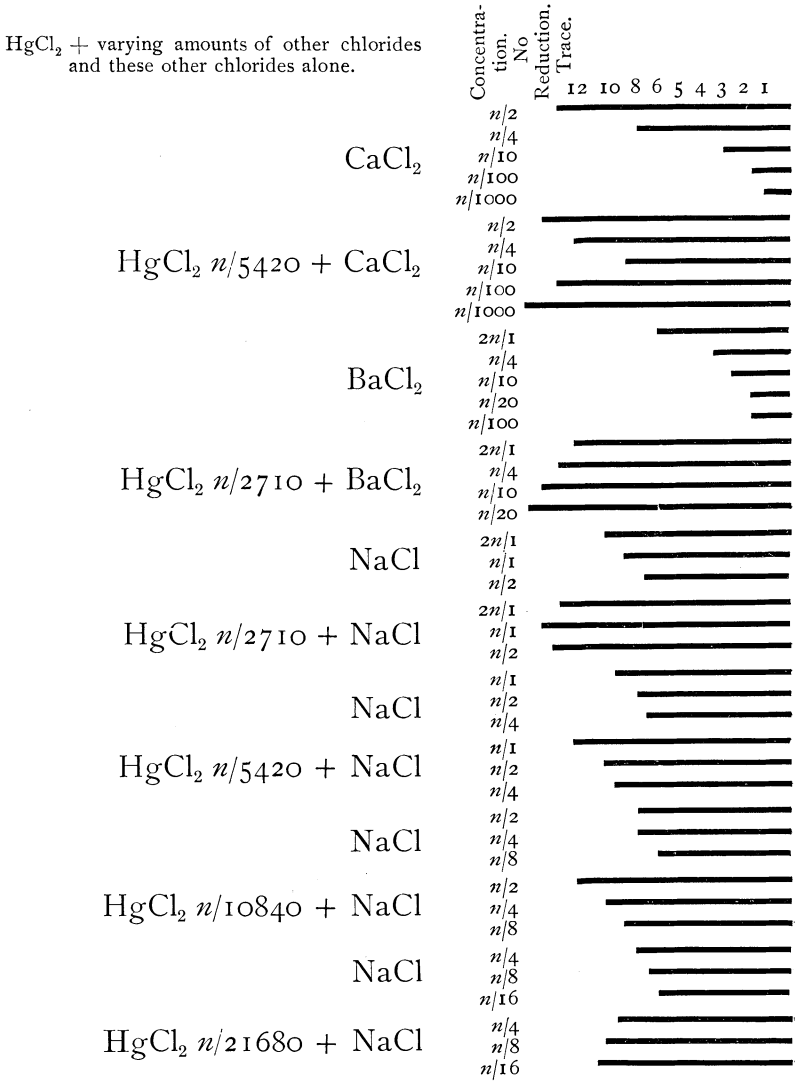


TABLE VI.

SHOWING THE EFFECTS OF ADDING VARIOUS OTHER CHLORIDES TO MERCURIC CHLORIDE



The concentration of mercuric chloride remains constant in each experiment, and to this varying concentrations of some other chloride are added.

The difference between the effect of the mercuric chloride + chloride and the check, containing no mercury, shows the effect of these other chlorides on the mercuric chloride.